Tissue Specific Knockout of the Cardiolipin Transacylase Enzyme TAFAZZIN in Both Liver and Pancreatic Beta Cells Protects Mice From Diet-Induced Obesity

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Abstract

Mutations in the TAFAZZIN gene result in the X-linked genetic disease Barth Syndrome. The protein product tafazzin is a transacylase enzyme that remodels the phospholipid cardiolipin with fatty acids. Some Barth syndrome boys exhibit a lean phenotype. In addition, whole body knockdown of tafazzin in mice protects them from diet-induced obesity through increased hepatic fatty acid oxidation and reduced basal insulin secretion. We thus hypothesized that tafazzin deficiency in both the liver and beta cells of the pancreas contribute to this lean phenotype. Through a Cre-Lox approach we generated control, liver-specific, pancreatic beta cell-specific and liver and pancreatic beta cell-specific double knockout male mice. The animals were fed a high fat diet for 8 weeks and body weight, liver weight and fat pad weights determined. Liver-specific or pancreatic beta cell-specific male tafazzin knockout mice accumulated weight gain (≃40% increase in body weight) at the same rate as control animals. In contrast, the liver- and beta cell-specific double tafazzin knockout mice exhibited a reduced rate of weight gain by 8 weeks (≃26% increase in body weight) compared to control or the single tafazzin knockout animals. In addition, at 8 weeks the double tafazzin knockout mice exhibited reduced weight gain in tissues known to accumulate fat including the liver, the gonadal, inguinal and perirenal white adipose tissues and the brown adipose tissue. Thus, liver- and pancreatic beta cell-specific double tafazzin knockout male mice are protected from high fat diet induced weight gain and fat accumulation. These results may partially explain why some Barth Syndrome boys exhibit a lean phenotype.

Introduction

Diet-induced obesity results in mitochondrial defects that contribute to development of tissue steatosis and metabolic complications[1]. Cardiolipin (CL) is a key mitochondrial lipid that regulates mitochondrial bioenergetics[2]. Alteration in CL is directly linked to development of obesity[1]. Barth Syndrome (BTHS) is a rare X-linked disorder in which the specific defect is a reduction in CL[3]. The loss in CL is caused by mutation in the TAFAZZIN gene which codes for tafazzin (Taz), a protein that synthesizes CL. Interestingly, BTHS boys possess a lean phenotype with mean weight in the 15th percentile and body mass index below the 5th percentile[4]. We previously showed that mice with whole body knockdown of Taz are protected from diet-induced obesity[5]. In addition, pancreatic islets isolated from Taz knockdown mice are protected from obesity-induced beta cell dysfunction[6]. The reduction in CL seen in most tissues of Taz knockdown mice was not mirrored in the liver[1] nor in pancreatic islets[6]. As a result, Taz knockdown mice exhibited normal hepatic mitochondrial function and, in combination with a reduction in basal insulin secretion from pancreatic islets, contributed to metabolic conditions consistent with a lean phenotype[5,6]. Recently, we demonstrated that treatment of high fat diet fed Taz knockdown mice with resveratrol, a small cardioprotective nutraceutical, further attenuated the aberrant lipid accumulation in their hearts[7]. Collectively, these studies identified a novel role for Taz in regulating susceptibility to diet-induced obesity. However, the mechanism by which Taz knockdown conferred protection to diet-induced obesity mediated liver and beta cell dysfunction was unclear since Taz was reduced in all tissues of these animals.

Based upon our previous observations[5-7], we hypothesized that a targeted deletion of Taz in both the liver and pancreatic beta cells in mice would protect them from diet-induced obesity and tissue steatosis. We utilized the Cre/lox system for generating tissue-specific knockout and our results show that liver- and pancreatic beta cell-specific double tafazzin knockout male mice are indeed protected from high fat diet-induced weight gain and fat accumulation.

Materials and Methods

Embyros from mice containing lox*P*-flanked Taz (flox) were previously generated by using sperm from a Taz flox male (Taz-fl/Y) for *in vitro* fertilization of wild type C57BL/6J oocytes (Dr. Douglas Strathdee, Beaton Institute, Glasgow, UK). The resulting heterozygous female (Taz-fl/+) and hemizygous males (Taz-fl/Y) were bred >9 times to a C57BL/6 background. To specifically delete Taz in the liver, mice homozygous/hemizygous for the floxed Taz allele are mated with B6.Cg-Speer6-ps1$^{Tg(Alb-cre)21Mgn}$/J strain of mice transgenic for cre recombinase under the albumin (*Albumin*) promoter (*Albumin*Cre). The B6.Cg-*Speer6-ps1*$^{Tg(Alb-cre)21Mgn}$/J strain of mice are obtained from Jackson Laboratories. Subsequent breeding took place between female mice heterozygous for the floxed Taz allele (Taz-fl/+, without *AlbuminCre*) with male mice which are wildtype (+/+) as well as male mice which are hemizygous for the floxed Taz allele (Taz-fl/Y) and heterozygous for the *AlbuminCre* transgene. This generated male and female mice which are hemizygous for the floxed Taz allele (Taz-fl/Y) and homozygous for the floxed allele (Taz-fl/Taz-fl) with *Albumin*Cre expression (liver specific knockout of Taz). Littermate controls included both male and female floxed Taz (Taz-fl/Y, Taz-fl/Taz-fl) without *Albumin*Cre expression (flox controls) and wildtype Taz

with *Albumin*Cre (cre recombinase controls) (**Figure 1**). The same protocol was used to generate pancreatic beta cell-specific TazKO mice driven under the Ins1 promoter using B6.Cg-$^{Tg(Ins1\text{-}cre/ERT)1Lphi}$/J strain of mice. In addition, liver- and pancreatic beta cell-specific double knockout mice were generated. 4 Month old male mice were fed a high-fat (23%, w/w) (Envigo Teklad, Rodent diet cat# TD.130261) diet for up to 8 weeks. Body weights were measured weekly and fat accumulating tissues were removed and weighed after 8 weeks. All data are presented as mean±standard error of the mean, n=2-4.

Results & Discussion

Control, liver-specific tafazzin knockout, pancreatic beta cell-specific tafazzin knockout, or liver- and pancreatic beta cell-specific tafazzin double knockout male mice were fed a high-fat diet for up to 8 weeks and body weights determined. Liver-specific or pancreatic beta cell-specific male tafazzin knockout mice accumulated weight gain (≃40% increase in body weight) at the same rate as control animals (**Figure 2A**). In contrast, the liver- and beta cell-specific double tafazzin knockout mice exhibited a reduced percentage of weight gain by 8 weeks (≃26% increase in body weight) compared to control or the single tafazzin knockout animals (**Figure 2B**). Thus, liver- and pancreatic beta cell-specific tafazzin double knockout male mice exhibit a reduced weight gain on a high fat diet.

We examined the overall body weight and weights of the tissues known to accumulate fat including the liver, the gonadal, inguinal and perirenal white adipose tissues (WAT) and the brown adipose tissue (BAT) in these animals. Liver-specific or pancreatic beta cell-specific male tafazzin knockout mice body weight was similar to

control animals (**Figure 3A**). In contrast, liver- and beta cell-specific double tafazzin knockout mice exhibited a lowered weight at 8 weeks compared to control or the single tafazzin knockout animals. In addition, at 8 weeks of feeding the high fat diet the double tafazzin knockout mice exhibited reduced weight gain in tissues known to accumulate fat including the liver, the gonadal WAT, inguinal WAT, perirenal WAT and BAT (**Figure 3B-F**).

Liver-specific or pancreatic beta cell-specific male tafazzin knockout mice body exhibited similar total gonadal WAT, inguinal WAT, perirenal WAT and BAT fat pad mass compared to control animals after 8 weeks of high fat diet (**Figure 4**). In contrast, liver- and beta cell-specific double tafazzin knockout mice exhibited lowered gonadal WAT, inguinal WAT, perirenal WAT and BAT fat pad mass compared to control or the single tafazzin knockout animals after 8 weeks of high fat diet. Thus, liver- and pancreatic beta cell-specific double tafazzin knockout male mice are protected from high fat diet induced weight gain and fat accumulation. In conclusion, we have found that liver and pancreatic beta cell Taz double knockout mice are protected from high fat diet-induced weight gain and fat accumulation. These findings complement our previous studies in which TAZ deficiency protects mice from diet-induced obesity[5-7]. Together, our data reveal a novel role for TAZ in regulating diet-induced weight gain and fat accumulation**.**

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Legend to Figures

Figure 1. Genotyping of TAFAZZIN knockout mouse liver. Primers for the Taz gene (TazGTKOU1 and TazGTFLoxD1) DNA were isolated from the liver of Floxed mice. Product size for WT Taz 3.1 kb. Product size for KO of Taz 280 bp. A representative gel is depicted.

Figure 2. A. Temporal increase in body weight of male mice fed the high fat diet. B. Percent increase in body weight after 8 weeks on the high fat diet (B). Control (Black), liver TazKO (Liver KO) (Red), pancreatic beta cell TazKO (B Cell KO) (Green) and liver- and pancreatic beta cell-specific double TazKO (Liver & Beta KO) (Yellow) male mice. Data represent mean±standard error of the mean, n=2-4.

Figure 3. Body weight (A), liver weight (B), gonadal WAT weight (C), inguinal WAT weight (D), perirenal WAT weight (E), and BAT weight in control (Black), liver TazKO (Liver KO) (Red), pancreatic beta cell TazKO (B Cell KO) (Green) and liver- and pancreatic beta cell-specific double TazKO (Liver & Beta KO) (Yellow) male mice fed a high fat diet for 8 weeks. Data represent mean±standard error of the mean, n=2-4.

Figure 4. Total fat pad weight in control (Black), liver TazKO (Liver KO) (Red), pancreatic beta cell TazKO (B Cell KO) (Green) and liver- and pancreatic beta cell-specific double TazKO (Liver & Beta KO) (Yellow) male mice fed a high fat diet for 8 weeks. Data represent mean±standard error of the mean, n=2-4.

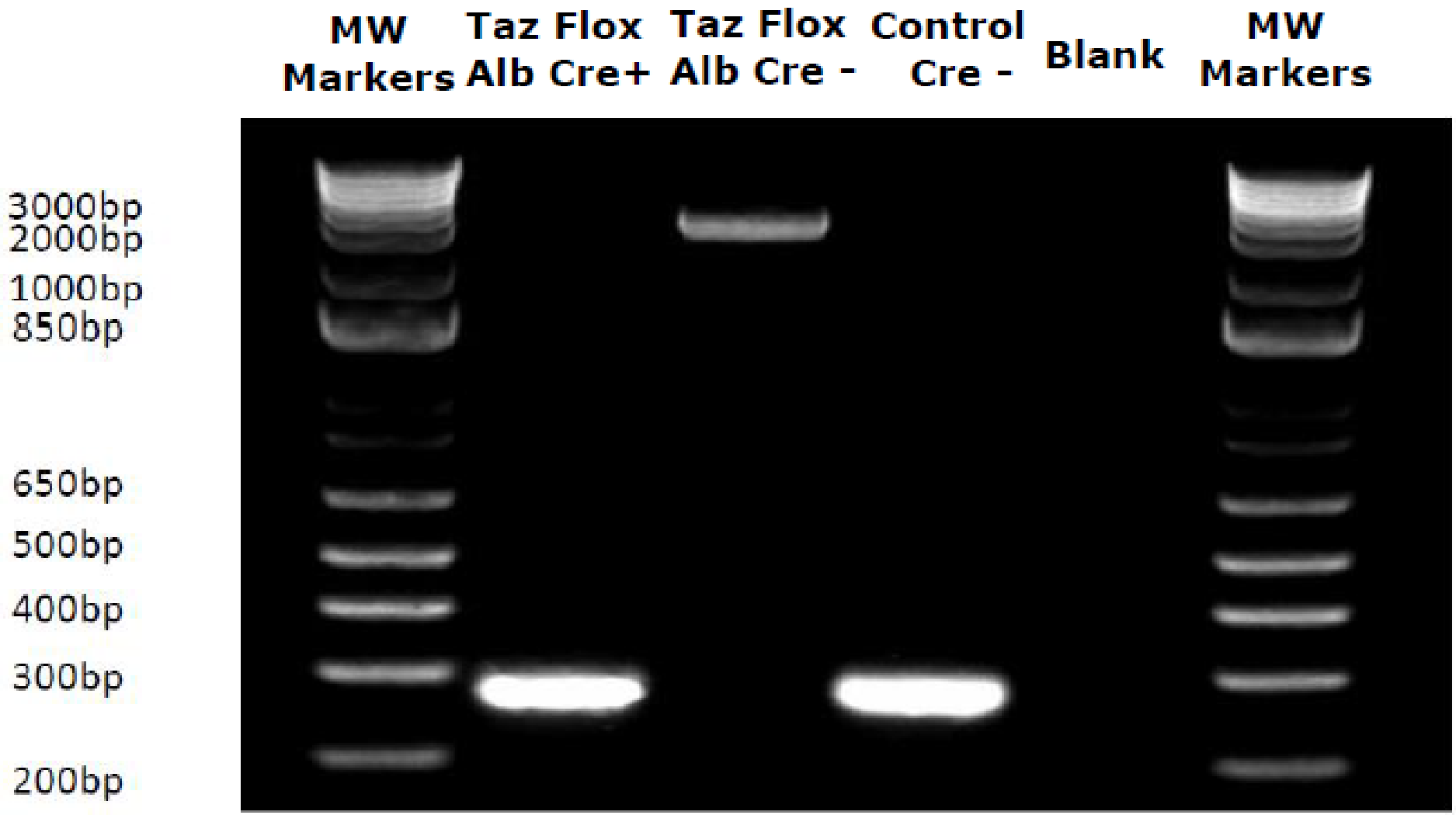
MW Markers
Taz Flox Alb Cre+
Taz Flox Alb Cre -
Control Cre -
Blank
MW Markers
3000bp
2000bp
1000bp
850bp
650bp
500bp
400bp
300bp
200bp

Figure 1

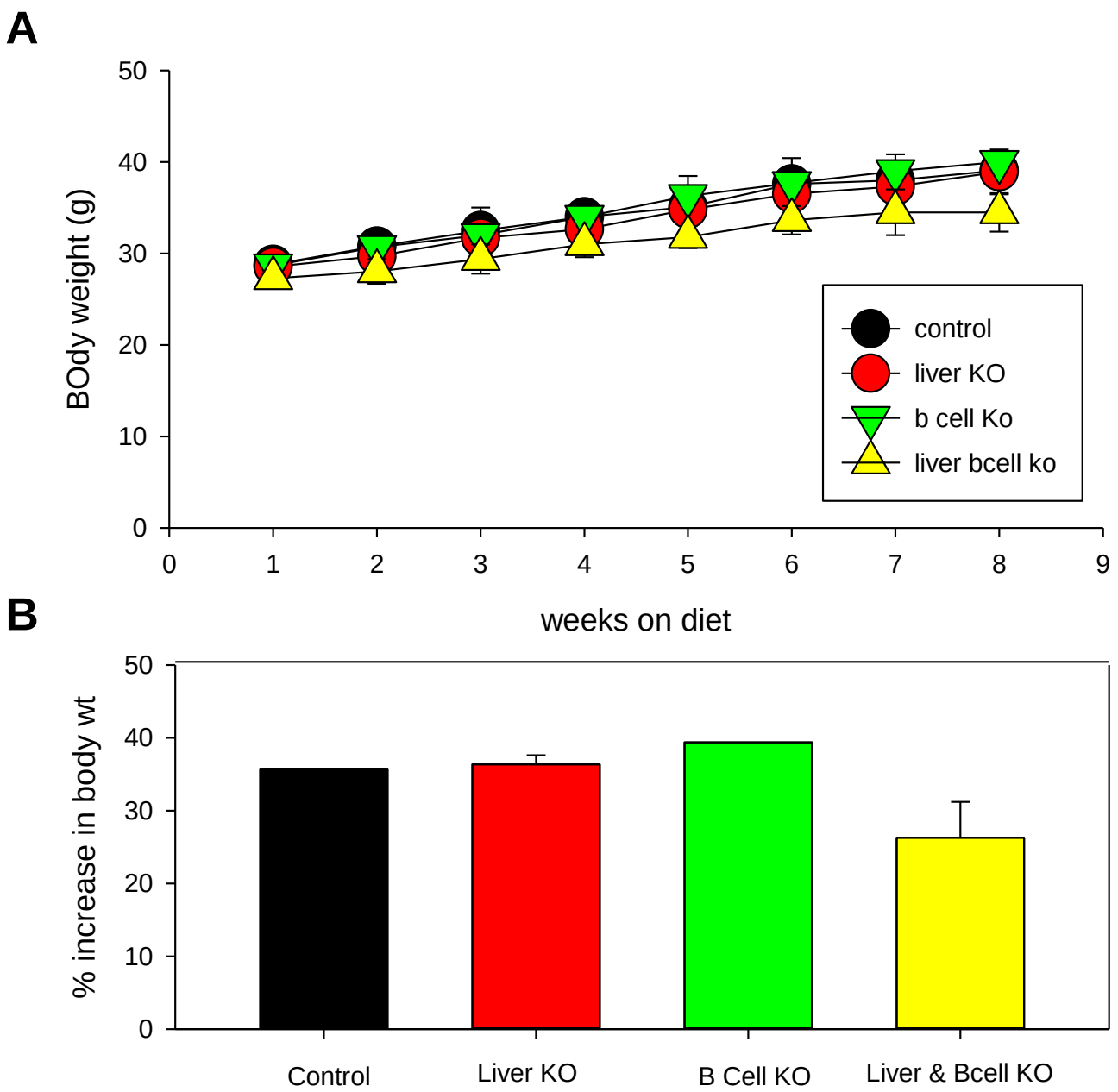
A
BOdy weight (g)
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
weeks on diet
control
liver KO
b cell Ko
liver bcell ko
B
% increase in body wt
50
40
30
20
10
0
Control
Liver KO
B Cell KO
Liver & Bcell KO

Figure 2

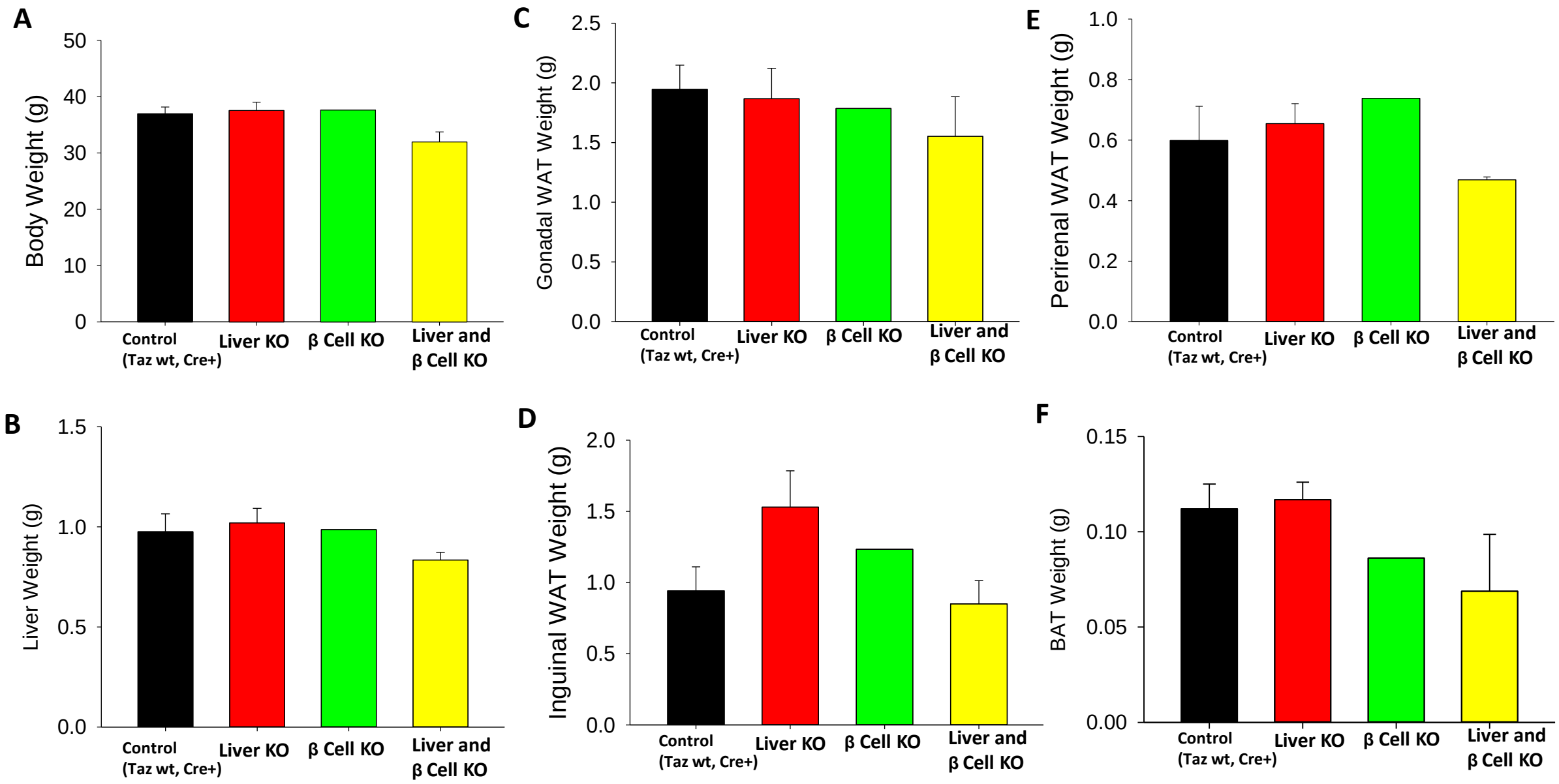
A
Body Weight (g)
50
40
30
20
10
0
Control (Taz wt, Cre+)
Liver KO
β Cell KO
Liver and β Cell KO
B
Liver Weight (g)
1.5
1.0
0.5
0.0
Control (Taz wt, Cre+)
Liver KO
β Cell KO
Liver and β Cell KO
C
Gonadal WAT Weight (g)
2.5
2.0
1.5
1.0
0.5
0.0
Control (Taz wt, Cre+)
Liver KO
β Cell KO
Liver and β Cell KO
D
Inguinal WAT Weight (g)
2.0
1.5
1.0
0.5
0.0
Control (Taz wt, Cre+)
Liver KO
β Cell KO
Liver and β Cell KO
E
Perirenal WAT Weight (g)
1.0
0.8
0.6
0.4
0.2
0.0
Control (Taz wt, Cre+)
Liver KO
β Cell KO
Liver and β Cell KO
F
BAT Weight (g)
0.15
0.10
0.05
0.00
Control (Taz wt, Cre+)
Liver KO
β Cell KO
Liver and β Cell KO

Figure 3

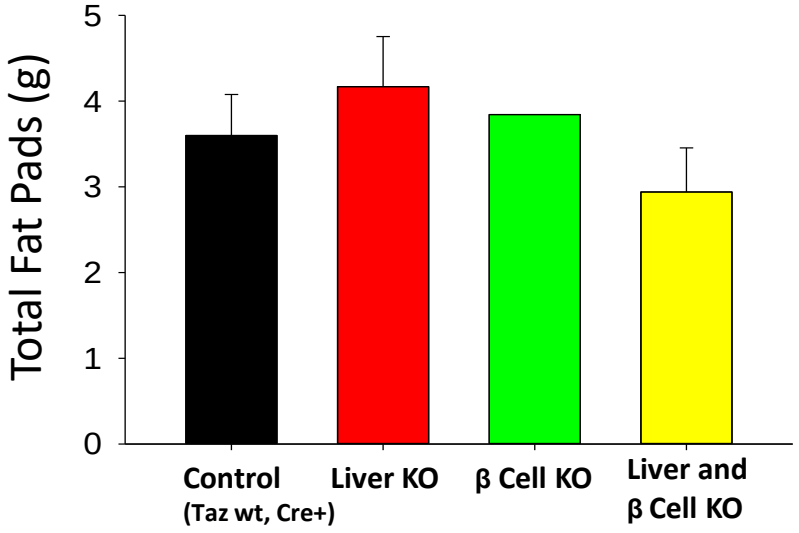
Total Fat Pads (g)
5
4
3
2
1
0
Control
(Taz wt, Cre+)
Liver KO
β Cell KO
Liver and
β Cell KO

Figure 4